MSD® Assay Development: Materials List and Preparation Guide

Reagent	Vendor	Materials	Size	Catalog Number
Blocker A Kit	MSD	Bovine Serum Albumin	250 mL	R93AA-2
			1000 mL	R93AA-1
Blocker A	MSD	Bovine Serum Albumin	15g	R93BA-4
Blocker B	MSD	Membrane Blocking Agent	1g	R93BB-1
			2g	R93BB-2
Blocker C	MSD	Casein Blocker	100 mL	R93BC-1
			1000 mL	R93BC-2
Blocker D-B	MSD	Bovine IgG	0.25 mL	R93BD-1
			1.2 mL	R93BD-2
Blocker D-G	MSD	Goat IgG	0.2 mL	R93BH-2
			1 mL	R93BH-3
Blocker D-M	MSD	Mouse Gamma Globulin	0.2 mL	R93BM-1
			1.8 mL	R93BM-3
Blocker D-R	MSD	Rabbit Gamma Globulin	0.2 mL	R93BR-2
			1 mL	R93BR-3
Inhibitor Pack	MSD	Phosphatase Inhibitor I, Phosphatase Inhibitor II, Protease Inhibitor	0.5 mL each	R70AA-1
Tris Lysis Buffer	MSD	Tris Buffer with non-ionic detergent	50 mL	R60TX-3
			200 mL	R60TX-2
Phosphatase Inhibitor I	Sigma	Phosphatase Inhibitor Cocktail 3	1 mL	P0044
Phosphatase Inhibitor II	Sigma	Phosphatase Inhibitor II	1 mL	P-5726
Protease Inhibitor	Thermo Scientific	Halt™ Protease Inhibitor Cocktail, EDTA-Free	1 mL	87785
Spin Columns	Thermo Scientific	Zeba™ Spin Desalting Columns, 40K MWCO	5 Pack	87768
			25 Pack	87769

Buffer Preparation Instructions

Tris Wash Buffer (10X)	500 mM Tris, pH 7.5 1.5 M NaCl 0.2% Tween-20 Store at 2-8ºC.
Tris Lysis Buffer (1X, Incomplete)	150 mM NaCl 20 mM Tris, pH 7.5 1 mM EDTA 1 mM EGTA 1% Triton-X-100 Store at 2-8ºC.

Buffer Preparation Instructions (continued)

Complete Lysis Buffer	10 mL 1X Tris Lysis Buffer 100 µL Phosphatase Inhibitor I 100 µL Phosphatase Inhibitor II 100 µL Protease Inhibitor Solution

Reagent Preparation Instructions

Blocker A	*General Notes:* Please refer to the Blocker A product insert, or individual assay inserts for preparation and storage information. *Storage:* Store at room temperature.
Blocker B	*General Notes:* Blocker B is provided as a dry solid and is required for the dilution of cell lysates only for certain targets. Please see individual assay inserts for specific preparation instructions. Blocker B solutions should be made fresh the day of use. Blocker B will appear milky and may not completely go into solution, this is normal. *Storage:* Store at room temperature.
Blocker C	*General Notes:* Blocker C is required for certain assays. Please see individual assay inserts for specific preparation. *Storage:* Store at 2-8°C.
Blocker D-B	*General Notes:* Blocker D-B is provided at a 10% stock concentration. The MSD working assay concentration is 0.7% for MSD Mouse Cytokine Assays, and 0.1% for MSD Alzheimer's Disease Assays or as indicated in individual assay protocols. *Storage:* Store at < -10°C.
Blocker D-G	*General Notes:* Blocker D-G is provided at a 10% stock concentration. The MSD working assay concentration is 0.1% or as indicated in individual assay protocols. *Storage:* Store at < -10°C.
Blocker D-M	*General Notes:* Blocker D-M is provided at a 2% stock concentration. The MSD working assay concentration is 0.1% or as indicated in individual assay protocols. *Storage:* Store at < -10°C.
Blocker D-R	*General Notes:* Blocker D-R is provided at a 10% stock concentration. The MSD working assay concentration is 0.1% or as indicated in individual assay protocols. *Storage:* Store at < -10°C.

Note:

It is important to use the blockers in accordance to the instructions of the individual protocol. Many D series blockers are added to the detection antibody diluent and not used as a traditional plate blocking solution.

Whole Cell Lysate Preparation Instructions

General notes:

All manipulations should be performed on ice. Prepare desired amount of Complete Lysis Buffer immediately prior to use. Lysis volumes will vary between cell types. Larger cells (such as NIH3T3, HeLa) should be lysed at concentrations of 1-5 x 10^6 cells per mL of Lysis Buffer. Smaller cells (such as Jurkat) should be lysed at concentrations of 1-5 x 10^7 cells per mL of Lysis Buffer. The Complete Lysis Buffer is optimal for many assays; however, any buffer that is compatible with traditional ELISA should be suitable for use in MSD assays. For phospho protein assays, phosphatase inhibitors are required in addition to the protease inhibitors. Phosphatase inhibitors are not required for non-phospho protein assays. In some cases it may be desirable to disrupt or denature the proteins in your samples by adding detergents, reducing agents, or urea. Please note that the addition of these reagents can adversely affect your assay if they are not diluted prior to adding sample to the plate. As a general rule final concentrations of non-ionic detergents e.g. Triton X-100 should not exceed 1%, final concentrations of ionic detergents e.g. SDS should not exceed 0.1%, and Final concentrations of reducing agents e.g. DTT or BME should not exceed 1 mM.

Reagents required:

Tris Lysis Buffer (1X, Incomplete)	1X Incomplete Tris Lysis Buffer
Complete Lysis Buffer	1X Complete Lysis Buffer ***NOTE:*** *For assays that require PMSF, a final concentration of 2 mM in Complete Lysis Buffer is recommended.* For 10 mL Complete Lysis Buffer, add 40 µL PMSF (from 500 mM stock prepared in DMSO) (Sigma, Catalog # P-7626). When adding PMSF to the buffer, all reagents should be at room temperature prior to mixing. The Complete Lysis Buffer should be mixed at room temperature on a rotator for 5 minutes (with no obvious precipitates), and should then be thoroughly chilled prior to use for lysate dilution or preparation.

Protocol:

Cells should be prepared as desired to activate target protein.

Suspension cells	Pellet cells (500 x g, 3 minutes at 2-8ºC) and wash one time with cold 1X PBS. Pellet cells again and resuspend in 1X Complete Lysis Buffer at 1-5 x 10^7 cells per mL. Incubate on ice for 30 minutes (a shorter incubation time of 15 minutes may be adequate for many targets). Centrifuge lysates at greater than or equal to 10,000 x g, 2-8ºC for 10 minutes to clear cellular debris from the lysate. Lysates can be quantitated using a detergent compatible protein assay such as BCA. Unused lysates should be aliquoted and snap frozen and stored at -80ºC.
Adherent cells	All volumes are determined for cells plated in 15 cm dishes. Remove media from the plates and wash cells one time with 5 mL cold 1 X PBS. Add 2 mL 1 X PBS to the plates and scrape the cells from the surface of the dish and transfer into 15 mL conical tubes. Pellet the cells at 500 x g for 3 minutes at 2-8ºC. Resuspend the cells in 0.5-2 mL of Complete Lysis Buffer per dish. Alternatively after medium removal, cells can be washed one time with PBS including a careful aspiration of residual PBS and lysed directly on the dish by adding 1-2 mL (depending on cell type) of Complete Lysis Buffer per dish. Incubate on ice for 30 minutes (a shorter incubation time of 15 minutes may be adequate for many targets). Centrifuge lysates at greater than or equal to 10,000 x g, 2-8ºC for 10 minutes to clear cellular debris from the lysate. Lysates can be quantitated using a detergent compatible protein assay such as BCA. Unused lysates should be aliquoted and snap frozen and stored at -80ºC.

Whole Cell Lysate Preparation Instructions (continued)

96 well format modifications	Successful adaptation to a 96 well format is cell type and target-dependent. The number of cells to be plated per well should be determined per cell type. General recommended plating concentrations for adherent cells range from 1 x 10^4-5 x 10^4 cells per well, and approximately 2 x 10^6 cells per mL (50-75 µL per well) for suspension cells. These numbers are provided as a guide and the optimal concentrations will vary depending upon cell line used.
Suspension cells	For flat bottom plates, experiments should be designed such that the final volume per well is 50-75 µL. Perform cell lysis using a 4X Complete Lysis Buffer concentrate, supplemented with protease and phosphatase inhibitors at 4X concentrations. Add 4X Complete Lysis Buffer directly to cells in the growth medium for a final 1X concentration in the well. NOTE: With some effort, a 10X Complete Lysis Buffer can also be prepared. *(For conical microwell plates, perform lysis by pelleting the cells, removing most of the growth medium and adding a constant amount of 1X Complete Lysis Buffer.)*
Adherent cells	Plate cells on biologically treated tissue culture ware to reduce variability due to cells lost as growth medium is removed. Treat cells as desired. Gently aspirate growth medium from microwell plate. A PBS wash step is not required and can introduce variability. Add 50-100 µL 1X Complete Lysis Buffer per well. Cell lysis time should be determined by the end user. Some targets are immediately available for detection, while others may require an incubation step at room temperature, 2-8ºC, or on ice with gentle agitation. Carefully pipet 25-90 µL cell lysate onto prepared capture plate. It is important to transfer a constant volume and avoid pipeting too vigorously, as the introduction of air bubbles may result.

Tissue Lysate Preparation Recommendations

The tissue pulverization technique described below uses a sonicator but a tissue grinder or an alternate tool can be used. You may use the MSD lysis buffer to generate homogenates or your own lysis buffer formulation. If there is a low amount of protein in your sample, a small amount of carrier protein such as 1% BSA may help.

Solid Tissues	Collect tissue on ice and rinse with ice cold PBS. Weigh tissue and keep it on ice as much as possible. Use 75 mg tissue with 0.5 mL ice cold lysis buffer. The ratio of lysis buffer to tissue may require additional optimization. We suggest adding double (2X) the protease inhibitors (and phosphatase inhibitors where required) to 1X MSD Tris Lysis Buffer (or other compatible lysis buffer). Using a probe sonicator set at 40% amplitude, sonicate tissue in three 30 sec bursts. Keep samples on ice. For best results, sonicate each sample once and replace it on ice, repeat this process until all samples have been sonicated three times. It is important to insure that during the sonication protocol the samples remain very cold. Centrifuge samples at 4°C for 10 minutes at 20,000 x g. Carefully remove supernatant and transfer to pre-chilled tubes (discard pellet) and determine the total protein concentration of each sample. Aliquot and snap freeze at -80°C.

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